

REMARKS

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Re:	Patent Application for Dale, et al	Dated:	January 6, 2006
Serial No.:		Art Unit:	
Filed:	July 8, 2003	Examiner	
For:	A Method of Selectively Producing Male or Female Sterile Plants	Action:	Preliminary Amendment

Please amend the specification and claims of the application as attached.
Please calculate the claim fees due in this application after including the changes of the preliminary amendment.

Please deduct any additional charges or credit any overpayments to account 07-0190. This is being provided in duplicate.

Respectfully submitted,



Dana Rewoldt, Reg. No. 33,762
Garst Seed Company
2369 330th Street, Box 500
Slater, IA 50244
(515) 685-5201

AMENDMENT TO THE SPECIFICATION

Page 1, Line 3, please insert the following paragraph:

This application claims foreign priority benefits under 35 U.S.C. §119 and/or § 365 of Great Britain application GB 0316040.5, filing date July 8, 2003 and Great Britain application GB 0401839.6, filing date January 28, 2004. This application also claims priority benefit under 35 U.S.C. §120 and/or § 363, and/or §365 for PCT international application PCT/GB2004/002447, filing date June 9, 2004.

AMENDMENT TO THE CLAIMS

1. (presently amended) A method of producing male or female sterile plants comprising the steps of transforming plant material with a polynucleotide which encodes at least one enzyme which reacts with a non-phytotoxic substance to produce a phytotoxic one, and regenerating the thus transformed material into a plant, wherein the said non-phytotoxic substance is applied to the plant up to the time of male or female gamete formation and/or maturation, so that the non-phytotoxic substance provides for the production of a phytotoxic one which selectively prevents the formation of or otherwise renders the said gametes non-functional, wherein the enzyme is expressed preferentially in either male or female reproductive structures and the non-phytotoxic substance is a D-alpha amino acid, or a peptide derivative of a non-protein D-alpha amino acid, characterised in that the enzyme is a mutant D-amino acid oxidase, obtainable from *Rhodotorula gracilis*, which oxidase comprises a lysine at position 58 rather than a phenylalanine in the wild type sequence.

2. (original) A method according to claim 1, wherein the said non-phytotoxic substance is applied in mixture along with at least one further substance which is selected from the group consisting of safeners, gametocides, glutathione-S-transferase inducers, cytochrome P450 inducers, herbicides, fertilizers, nematocides, synergists, insecticides, fungicides, hormones, plant-growth regulators and cytochrome P450 inhibitors.

3. (Presently amended) A method according to claim 1 or 2, wherein the non-phytotoxic substance is applied foliarly and is a phloem mobile and metabolically stable oxidisable substrate of the enzyme, wherein the enzyme provides the phytotoxic product, as a direct or indirect one from the non-phytotoxic substance.

4. (Presently amended) A method according to the preceding claim 3, wherein the phytotoxic product is an indirect one produced in the form of peroxide and/or a super oxide anion.

5. (Presently amended) A method according to claim ~~either of claims 3 or 4~~, wherein the non-phytotoxic substance is D-aspartate or D-glutamate and the enzyme oxidises the said amino acid to a 2-keto acid with concomitant reduction of oxygen to a peroxide anion.
6. (Presently amended) A method according to ~~any preceding~~ claim 1 wherein the enzyme comprises substitutions at positions 213, 223 and/or 238 when compared to the wild type sequence.
7. (Presently amended) A method according to ~~the preceding~~ claim 6, wherein the oxidase has at position 213 an amino acid selected from the group consisting of His, Ser, Thr, Cys, Gln, Gly, Asn and Ala, and/or at position 238 an amino acid selected from the group consisting of His, Ser, Thr, Cys, Asn, Gln, Gly and Ala, and/or at position 223 an amino acid selected from the group consisting of His, Ser, Thr, Cys, Ala, Gly, Gln and/or Asn.
8. (Presently amended) A method according to ~~the previous~~ claim 7 where the amino acid at position 213 is Ser or Thr.
9. (Presently amended) A method according to claim ~~any one of claims 3[8]~~, wherein the enzyme is targeted to other than the peroxisome.
10. (Presently amended) A method according to claim 1 ~~or 2~~, wherein the non-phytotoxic substance is either the D enantiomer of phosphinothricin or a D enantiomer of bialaphos.
11. (Presently amended) A method according to claim ~~either of claims 1 or 2~~, wherein the non-phytotoxic substance is comprised within a mixture, which contains a phytotoxic substance and wherein the enzyme oxidises an amino acid to a 2-keto acid with concomitant reduction of oxygen to a peroxide anion.

12. (Presently amended) A method according to claim 11 ~~the preceding claim~~ wherein the enzyme is a mutant D-amino acid oxidase obtainable from *Rhodotorula gracilis* which oxidase comprises substitutions at positions 213 and/or 238 and/or 223 when compared to the wild type sequence, or is a D-aspartate oxidase.

13. (Presently amended) A method according to claim 12 ~~the preceding claim~~, wherein the oxidase obtainable from *Rhodotorula* has at position 213 an amino acid selected from the group consisting of: His, Ser, Thr, Cys, Gln, Gly, Asn and Ala, and/or at position 238 an amino acid selected from the group consisting of His, Ser, Thr, Cys, Gln, Gly, Asn and Ala, and at position 223 an amino acid selected from the group consisting of: His, Ser, Thr, Cys, Gln, Gly, Asn and Ala.

14. (Presently amended) A method according to ~~the previous claim~~ claim 13 where the amino acid at position 213 is Ser or Thr.

15. (Presently amended) A method according to claim ~~any one of claims 10[13]~~, wherein the mixture comprises both D and L phosphinothricin and the plant material expresses a PAT gene substantially only in green tissues and/or in floral tissue which produce gametes being other than those that are rendered non-functional.

16. (original) A mutant D-amino acid oxidase obtainable from *Rhodotorula gracilis*, capable of oxidising phosphinothricin, which comprises a lysine at position 58 rather than a phenylalanine in the wild type sequence.

17. (original) An oxidase according to claim 14, further comprising amino acid substitutions in at least one position selected from the group consisting of 213, 223, 238.